Brooks, R. V. & Klyne, W. (1957). *Biochem. J.* **65**, 663.
Bush, I. E. (1952). *Biochem. J.* **50**, 370.
Forchielli, E., Brown-Grant, K. & Dorfman, R. I. (1958). *Proc. Soc. exp. Biol., N.Y.*, **99**, 594.
Fotherby, K. (1958). *Biochem. J.* **69**, 596.
Fotherby, K. (1959). *Biochem. J.* **71**, 209.
Fotherby, K., Colás, A., Atherden, S. M. & Marrian, G. F. (1956). *Biochem. J.* **64**, 50P.
Fotherby, K., Colás, A., Atherden, S. M. & Marrian, G. F. (1957). *Biochem. J.* **66**, 664.
Grant, J. K. (1960). *Symp. biochem. Soc.* **18**, 24.
Hagopian, M. & Levy, L. K. (1958). *Biochim. biophys. Acta*, **30**, 641.
Kaplan, N. O., Colowick, S. P. & Neufeld, E. F. (1952). *J. biol. Chem.* **195**, 107.
Leybold, K. & Staudinger, H. (1959). *Biochem. Z.* **331**, 389.
Merrill, R. C. (1958). *Physiol. Rev.* **38**, 463.
Neher, R., Desaulles, P., Vischer, E., Wieland, P. & Wettstein, A. (1958). *Helv. chim. acta*, **41**, 1667.
Neher, R., Meystre, Ch. & Wettstein, A. (1959). *Helv. chim. acta*, **42**, 132.
Neher, R. & Wettstein, A. (1960). *Helv. chim. acta*, **43**, 1628
Okada, M., Fukushima, D. K. & Gallagher, T. F. (1959). *J. biol. Chem.* **234**, 1688.
Rao, B. G. & Heard, R. D. H. (1957). *Arch. Biochem. Biophys.* **66**, 504.
Rubin, B. L. (1957). *J. biol. Chem.* **227**, 917.
Ryan, K. J. (1958). *Endocrinology*, **63**, 392.
Ryan, K. J. (1959). *J. biol. Chem.* **234**, 2006.
Samuels, L. T. (1960). In *Metabolic Pathways*, **1**, p. 431. Ed. by Greenberg, D. M. New York: Academic Press Inc.
Schneider, J. J. & Mason, H. L. (1948). *J. biol. Chem.* **172**, 771.
Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (editors) (1957). *Manometric Techniques*, 3rd ed. Minneapolis: Burgess Publishing Co.
Vogel, A. I. (1951). *A Text-book of Practical Organic Chemistry including Qualitative Organic Analysis*, 2nd ed. London: Longmans, Green and Co.
Weissberger, A., Proskauer, E. S., Riddick, J. A. & Toops, E. E., jun. (1955). *Organic Solvents*, 2nd ed. New York: Interscience Publishers, Inc.

Biochem. J. (1962) **82**, 394

The Monosaccharide Units in Specific Glycolipids of *Mycobacterium avium*

By A. P. MacLennan
Microbiological Research Establishment, Porton, near Salisbury, Wilts.

(*Received* 28 *July* 1961)

Lipids specific for species or type have been discovered in ethanol–ether extracts of strains of *Mycobacterium avium*, *Mycobacterium bovis* and the photochromogenic group (Runyon, 1955, 1959) of 'atypical' mycobacteria (Randall & Smith, 1953; Smith, Randall, Gastambide-Odier & Koevoet, 1957; Smith, Randall, MacLennan, Putney & Rao, 1960*b*). The specific substances were separated from non-specific lipids by column-adsorption chromatography and recognized by their distinctive infrared spectra. They were subsequently found to be glycolipids containing, characteristically, 6-deoxyhexoses and their methyl ethers (MacLennan, Smith & Randall, 1960, 1961; Smith *et al.* 1960*b*). The general name of 'mycoside' has been proposed (Smith, Randall, MacLennan & Lederer, 1960*a*) for this class of substances.

Mycoside A, characteristic of photochromogenic mycobacteria, contains 2-*O*-methylfucose, 2-*O*-methylrhamnose and 2,4-di-*O*-methylrhamnose; mycoside B, specific for *M. bovis*, contains 2-*O*-methylrhamnose alone (MacLennan *et al.* 1960, 1961). The lipid moieties of these substances contain an aromatic alcohol in ester linkage with branched-chain fatty acids (Smith *et al.* 1957, 1960*a*, *b*).

Mycoside C, produced by *M. avium*, differs from other mycosides in that it contains a peptide (Smith *et al.* 1960*a*, *b*). It now appears that mycoside C is a mixture of peptido-glycolipids which can be resolved by adsorption chromatography into a number of components differing in their content of carbon, nitrogen and sugars, and in their optical activity (Jollès, Bigler, Gendre & Lederer, 1961).

The present study describes the isolation and identification of the sugars present in the glycolipid mixture, mycoside C, and in one of its components, mycoside C_1, recently isolated and characterized by Jollès *et al.* (1961).

The analysis of a lipopolysaccharide antigen of *Pseudomonas pseudomallei* is also described since it appeared (Chambon, Bourdet & Staub, 1957) that this substance might contain one or more of the uncommon sugars hitherto found only in bacteria of the order Actinomycetales.

MATERIALS AND METHODS

The samples of mycoside C employed were prepared and purified by Professor H. M. Randall (Department of Physics, University of Michigan, Ann Arbor, Mich., U.S.A.), Dr D. W. Smith (Department of Medical Microbiology, University of Wisconsin, Madison 6, Wis., U.S.A.) and Professor E. Lederer (Institut de Biologie Physicochimique, Paris). References will be quoted for those materials whose preparation has been fully described elsewhere.

Bacteria. The strains of *M. avium* employed were 802 (Institut Pasteur), Faisan I, II, III and IV (supplied to D. W. Smith by E. Lederer), 4133–74 (Feldman), and 297R_1, one colonial form isolated from 4133–74 (D. W. Smith, personal communication). Strain 3327 is a chromogenic mycobacterium (Prissick & Masson, 1957). Strains P3, P7 and P23 have been described as non-photochromogenic mycobacteria (Runyon, 1955, 1959); their close resemblance to *M. avium* has been commented on by several workers (see Bojalil & Cerbon, 1960, for references).

Cultivation of bacteria. The method of cultivation of strain 802 has been described by Jollès *et al.* (1961). All other strains were cultivated by D. W. Smith on a malic acid medium (Wong & Weinzirl, 1936) at 37° in static culture for periods of from 5 to 12 weeks for different organisms.

Mycoside C preparations

Five preparations, *A–E*, were used.

Preparation A. This, obtained from strain 802, was a sample (103 mg.) of fraction 7, chromatogram III, the preparation of which is described by Jollès *et al.* (1961).

Preparation B. This was a pool of fraction 9, chromatogram II (283 mg.) and fractions 9, 10, chromatogram III (333 mg.); see Jollès *et al.* (1961).

Preparations C–E. These were pools of mycoside C preparations from a number of strains. They were prepared by D. W. Smith and H. M. Randall from ethanol–ether extracts of cells (Smith *et al.* 1960*b*) by chromatography on Magnesol–Celite and silicic acid–Hyflo Supercel columns (Magnesol is synthetic magnesium trisilicate, supplied by Westvaco Chlorine Products, South Charleston, W.V., U.S.A.). Details of the chromatographic process, including the nature and sequence of eluting solvents, are given by Smith *et al.* (1960*b*). Mycoside C was eluted from Magnesol columns by diethyl ether–methanol (80:20, v/v), from silicic acid columns by chloroform–methanol (80:20, v/v). The criterion employed in making the pools *C–E* was that the individual samples had the infrared spectrum characteristic of mycoside C.

Preparation C. This was pooled preparations (400 mg.) from strains Faisan I, II, III and IV.

Preparation D. This was pooled preparations (430 mg.) from strains Faisan I–IV, P 23 and 3327.

Preparation E. This was pooled preparations (83 mg.) from strain 4133–74 and its derivative 297R_1.

Paper chromatography and electrophoresis

Chromatography was as described by MacLennan *et al.* (1961). The solvent systems used were: solvent A, the upper phase of butan-1-ol–ethanol–water (4:1:5, by vol.); solvent B, butan-1-ol–pyridine–water (6:4:3, by vol.); solvent C, phenol-rich phase of aq. 90% (w/v) phenol.

The technique for electrophoresis in borate buffer was as described by Foster (1952) and MacLennan *et al.* (1961).

EXPERIMENTAL AND RESULTS

Fig. 1 shows the similarity in the infrared spectra of mycoside C preparations *B–E*.

Hydrolysis of mycoside C. Preparations *B–E* were hydrolysed separately with N-sulphuric acid for 16 hr. at 100° in sealed ampoules. The insoluble lipid residues after hydrolysis were rehydrolysed separately under identical conditions. A third hydrolysis of the insoluble material did not liberate detectable amounts of sugar. The acid-soluble fractions from the first and second hydrolyses of each preparation were washed with chloroform, neutralized with barium hydroxide, centrifuged and filtered to remove barium sulphate, and finally evaporated to dryness *in vacuo* over phosphorus pentoxide.

Samples of the eight hydrolysates were examined by conventional paper chromatography. On chromatograms developed with solvent A, the *p*-anisidine hydrochloride reagent (Hough, Jones & Wadman, 1950) revealed eight sugar spots in the first hydrolysates. Of these, four to five were detected also in the second hydrolysates but additional spots were not detected. Preparations *B–E* thus appeared to be identical with respect to the number and nature of sugars present but there were quantitative differences between the preparations with respect to individual sugars, and in each of the preparations there was variation in the amounts of the different sugars present.

The eight sugars found in preparations *B* and *E* arise from the mycoside C of two individual strains of *M. avium*. The absence of additional sugars in preparations *C* and *D*, which each contained the products of a number of strains, suggests the absence of strain specificity with respect to the sugar components of mycoside C, but it must be recognized that some components of the pools may not have contained all of the sugars found in the pools.

The separate hydrolysates were combined and chromatographed as bands on sheets of Whatman no. 1 paper that had been washed with water as described by MacLennan *et al.* (1961). The chromatograms were developed with solvent A and the bands detected by spraying marker strips with *p*-anisidine hydrochloride. Each band was eluted with water and the eluate evaporated to dryness *in vacuo*. In those cases where chromatographic examination showed the presence of slight contamination with other sugars the material was purified by chromatography as described above.

Identification of the sugars

It will be convenient to refer to the mycoside C sugars as S 1–S 8, in order of increasing R values.

S 3–S 8 reacted as 6-deoxyhexoses in the 'CyR10' sulphuric acid–cysteine reaction of Dische & Shettles (1948); the narrow specificity of this test has been discussed elsewhere (Dische & Shettles,

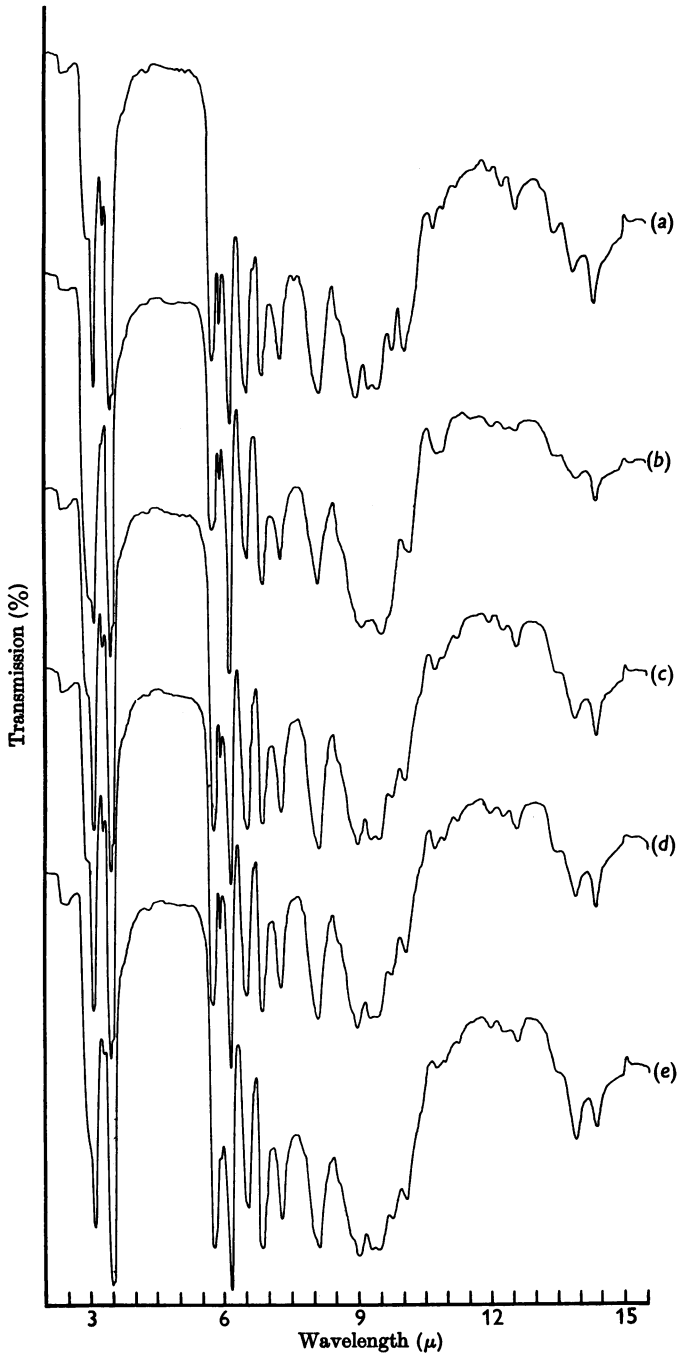

Fig. 1. Infrared-absorption spectra of mycoside C preparations; Nujol mull. (*a*), preparation A; (*b*), preparation *B*; etc. The spectra are displaced vertically.

1948; MacLennan *et al.* 1961). S 3 and S 4 were also tested with a vanillin–perchloric acid spray reagent (Godin, 1954; MacLennan, Randall & Smith, 1959) and found to give the rapidly fading orange–red colour characteristic of 6-deoxyhexoses. S 5–S 8 were found to be methyl ethers of 6-deoxyhexoses; as a first step in identification it was necessary to demethylate with boron trichloride (Allen, Bonner, Bourne & Saville, 1958; MacLennan *et al.* 1961) and thus identify the parent sugar.

The eight structural isomers of the 6-deoxyhexose series can be distinguished one from another by the combined methods of paper chromatography and paper electrophoresis in borate buffer (MacLennan *et al.* 1961). S 3 and S 4, and the parent sugars of S 5–S 8 were identified in this way.

The R_{rhamnose} values of the methylated sugars of mycoside A are recorded in Table 1.

S 1 *and* S 2. These were identified as glucose and arabinose, respectively, by their colour reactions with *p*-anisidine hydrochloride (Hough *et al.* 1950) and their failure to separate from authentic samples of glucose and arabinose on paper chromatography (solvent B) and paper electrophoresis. Arabinose is separated clearly from xylose, lyxose and ribose by both methods.

S 3. This was identified as rhamnose.

S 4. This was identified as 6-deoxytalose. In support of this identification, S 4 and authentic 6-deoxytalose reacted intensely on chromatograms sprayed with a triphenyltetrazolium chloride–sodium hydroxide reagent (Trevelyan, Procter & Harrison, 1950), whereas rhamnose and fucose gave relatively weak reactions. It might be suggested that S 4 could be a monomethyl ether of a 6-deoxyhexose, having electrophoretic and chromatographic properties identical with those of 6-deoxytalose. This possibility is rendered very unlikely by the fact that attempts to demethylate S 4 with boron trichloride yielded only unchanged S 4. Moreover, the introduction of *O*-methyl groups into a sugar reduces its paper-electrophoretic mobility in borate buffer to an extent that depends upon the number and position of the substituent groups (Foster, 1953). Since S 4 does not separate from 6-deoxytalose on paper electrophoresis it could therefore only be an *O*-methyl derivative of sugars with higher mobilities, namely fucose, 6-deoxyaltrose and 6-deoxyidose (MacLennan *et al.* 1961). However, on paper chromatography, where *R* values of sugars are increased by *O*-methylation (Hirst & Jones, 1949; Gardiner & Percival, 1958), S 4 could be distinguished from both 2- and 3-*O*-methylfucose (Table 1), and hence from other methyl ethers of the fucose series (see Gardiner & Percival, 1958). The remaining possibility, that S 4 is an ether of 6-deoxyaltrose or 6-deoxyidose, is rendered unlikely by the observation (Krauss, Jäger, Schindler & Reichstein, 1960) that the 3-*O*-methyl derivatives of these sugars have higher *R* values than 3-*O*-methylrhamnose on paper chromatograms, whereas S 4 was found to have a lower *R* than 3-*O*-methylrhamnose (Table 1). In at least two series of 6-deoxyhexose ethers, those of rhamnose and fucose, the 3-*O*-methyl derivative has the lowest *R* of the series (Table 1, and see Gardiner & Percival, 1958).

S 5. The products of boron trichloride treatment were rhamnose and unchanged S 5. Paper chromatography in solvent A and paper electrophoresis did not separate S 5 from mixtures with authentic samples of 3-*O*-methyl-L-rhamnose (acofriose) and both sugars gave a green–brown colour with *p*-anisidine hydrochloride. S 5 was readily distinguished from 2-*O*-methylrhamnose (red–brown with *p*-anisidine) by both methods and by its positive reaction with a triphenyltetrazolium chloride–sodium hydroxide spray, a reagent which does not react with C-2 substituted sugars (see Gardiner & Percival, 1958). Moreover, S 5 could not be a dimethyl ether of rhamnose, as shown by direct chromatographic (Table 1) and electrophoretic comparison. Nor is it likely to be either 4-*O*-methylrhamnose, which has a higher *R* than acofriose in a solvent similar to solvent A (Andrews, Hough & Jones, 1955) or 5-*O*-methylrhamnose, which can be presumed to have a higher *R* in the light of behaviour in the fucose ether series (Gardiner & Percival, 1958).

S 6. Demethylation with boron trichloride gave only 6-deoxytalose and unchanged S 6. Of the 6-deoxytalose methyl ethers only the 3-methyl ether (acovenose) was available for comparison. S 6 could not be distinguished from acovenose by paper chromatography in solvent A or by paper electrophoresis. The intense reaction of S 6 with triphenyltetrazolium chloride–sodium hydroxide confirmed both the absence of a C-2 substituent and the presence of 6-deoxytalose as the parent sugar.

S 7. Insufficient sugar was available for de-

Table 1. R_{rhamnose} *values of methyl ethers of* 6-*deoxyhexoses in butan*-1-*ol–ethanol–water* (4:1:5, *by vol.; upper phase*)

Sugar	R_{rhamnose}
3-*O*-Methylfucose	1·18
6-Deoxytalose (S 4)	1·24
2-*O*-Methylfucose	1·32
3-*O*-Methylrhamnose (S 5)	1·42
2-*O*-Methylrhamnose	1·48
3-*O*-Methyl-6-deoxytalose (S 6)	1·70
2,3-Di-*O*-methylrhamnose (S 7)	1·90
2,4-Di-*O*-methylrhamnose	1·96
3,4-Di-*O*-methylrhamnose (S 8)	2·04

methylation and the sugar could not therefore be conclusively identified. S 7 did not react with triphenyltetrazolium chloride–sodium hydroxide, showing the presence of a C-2 substituent. The *R* of S 7 in solvent A was much greater than that of 2-*O*-methylrhamnose (Table 1). Direct comparison with authentic samples of 2,3-, 2,4- and 3,4-di-*O*-methylrhamnose by paper chromatography and paper electrophoresis, under conditions which permit the three dimethyl ethers to be distinguished one from another (Butler, Lloyd & Stacey, 1955), showed S7 to be indistinguishable from 2,3-di-*O*-methylrhamnose. The red–brown colour with anisidine was distinct from the green–brown given by the 2,4- and 3,4-ethers.

It is most unlikely that S7 is 2-*O*-methyl-6-deoxytalose since its mobility on paper electrophoresis is less than that of 2-*O*-methylrhamnose, whereas the 6-deoxytalose derivative can be presumed to have a higher mobility from consideration of the order of separation of the parent 6-deoxyhexoses on paper electrophoresis (MacLennan *et al.* 1961). Nor is it likely that S 7 is a 2:*x*-di-*O*-methyl-6-deoxytalose since it has an *R* on paper chromatography in solvent A only slightly greater than 3-*O*-methyl-6-deoxytalose, whereas the dimethyl ethers of rhamnose and fucose have *R* values much greater than their 3-*O*-methyl ethers (Table 1; Gardiner & Percival, 1958).

S 8. This sugar formed white crystals of a waxy consistency when eluted from chromatograms with water and evaporated to dryness. The products of boron trichloride treatment were unchanged S 8, rhamnose and two other sugars. One of these could not be distinguished from 3-*O*-methylrhamnose by paper chromatography in solvent A or by paper electrophoresis. It was detected as a separate spot only on chromatograms since on paper electrophoresis it did not separate from unchanged S 8. The fourth component of the demethylation products had an *R* slightly higher than 3-*O*-methylrhamnose on chromatograms in solvent A, suggesting that it could be 4-*O*-methylrhamnose (Andrews *et al.* 1955).

In support of the results of the demethylation S8 was indistinguishable from 3,4-di-*O*-methylrhamnose in its colour reaction with anisidine and in its behaviour on paper electrophoresis and paper chromatography. It was further distinguished from 2,3- and 2,4-di-*O*-methylrhamnose by a positive reaction with triphenyltetrazolium chloride–sodium hydroxide, and again from the 2,4-ether by a positive reaction with a periodate–benzidine spray (Cifonelli & Smith, 1954). S 8 had m.p. 86–88°; 3,4-di-*O*-methylrhamnose has a reported m.p. 91–92° (Butler *et al.* 1955) (Found for S8: OMe, 30·5. A di-*O*-methyl-6-deoxyhexose, $C_6H_{16}O_5$, requires OMe, 32·3%).

Analysis of preparation A

Preparation *A* contained 33–35% of 6-deoxyhexose (as L-rhamnose) measured by the sulphuric acid–cysteine reaction (Dische & Shettles, 1948; MacLennan *et al.* 1961).

By the methods of hydrolysis, sugar isolation and characterization described above, four sugars were isolated and identified as 6-deoxytalose, 3-*O*-methyl-6-deoxytalose, 3,4-di-*O*-methylrhamnose and 3-*O*-methylrhamnose, the last being present in small amount. Glucose, arabinose, rhamnose and 2,3-di-*O*-methylrhamnose were not detected.

Glycolipid of strain P 3

P 3 is classified as a non-photochromogenic mycobacterium (Runyon, 1955, 1959).

The ethanol–ether-extracted lipid of P 3 was chromatographed on Magnesol–Celite and the material eluted by ether–methanol (8:2, v/v) was rechromatographed on silicic acid–Hyflo Supercel. The fraction eluted by chloroform–methanol (8:2, v/v) yielded, on hydrolysis, glucose, xylose, rhamnose and a 6-deoxyhexose derivative with an *R* similar to that of 2-*O*-methylrhamnose in solvent B. This chloroform-soluble glycolipid gave an opalescent solution in water. The infrared spectrum was distinct from those of mycosides A, B and C. Infrared absorption bands due to peptide were not detected and amino acids were not found in 6N-hydrochloric acid hydrolysates.

Unfortunately, the culture of P 3 from which the glycolipid was isolated may have undergone serious population changes during its long period of cultivation since two distinct colonial forms were subsequently isolated from it (D. W. Smith, personal communication). Although the glycolipid is of interest since it is distinct from the better characterized mycosides A, B and C, and contains, apparently, a preponderance of unmethylated sugars, including xylose, the nature of the organism producing it is in doubt and will be established only by further study.

The 'K' antigen of Pseudomonas pseudomallei

Chambon *et al.* (1957) detected 'fast-moving' sugars on paper chromatograms in their examination of the composition of a lipopolysaccharide 'K' antigen extracted from a strain of *P. pseudomallei*. The major unidentified sugar component of this antigen reacted as a 6-deoxyhexose with the sulphuric acid–cysteine reagent of Dische & Shettles (1948). At the suggestion of Dr Staub and by using her preparation of K antigen this sugar was isolated and examined.

A sample (8 mg.) was hydrolysed as described above. Lipid (1·8 mg.) was recovered from the hydrolysate by chloroform extraction. The neutral-

ized hydrolysate was evaporated to dryness and the sugar syrup examined by paper chromatography in solvents A and C and by paper electrophoresis. The principal 'fast-moving' sugar was identified as 6-deoxytalose by virtue of its *R* values and strong reaction with triphenyltetrazolium chloride–sodium hydroxide. The positive reaction with sulphuric acid–cysteine reported by Chambon *et al.* (1957) was confirmed on a sample of the sugar eluted with water from an electrophoretogram. A second faint component of high *R* was detected in the hydrolysate. This could not be distinguished from 3-*O*-methyl-6-deoxytalose by paper chromatography (solvent A) and paper electrophoresis, but it was not possible to obtain further evidence supporting this very tentative identification. As reported by Chambon *et al.* (1957), glucose was the other major component of the K antigen and traces of galactose and rhamnose were detected.

DISCUSSION

The preparations of mycoside C examined were largely pools of materials isolated individually from a number of strains of bacteria, including some whose precise relationship to *M. avium* is not yet established. It is clear, nevertheless, from the known composition of the pools, that the eight sugars isolated were present in the mycoside C of the avian strain 802 and in that of the avian strain 4133–74, or its derivative 297 R_1. The same eight sugars were found in the other pools examined but it remains possible that not all the mycoside C preparations in these pools contained the eight sugars. The chromatographic results suggested quantitative variation among and within the preparations with respect to their sugar components.

These observations must be considered in the light of the demonstration by Jollès *et al.* (1961) that the mycoside C of the avian strain 802 is a mixture of peptido-glycolipids which can be separated by chromatography. One of these glycolipids, very closely related to preparation *A* (see text and Jollès *et al.* 1961), contained only 6-deoxytalose, 3-*O*-methyl-6-deoxytalose and 3,4-di-*O*-methylrhamnose. Jollès *et al.* (1961) have assigned a provisional formula to this compound, mycoside C_1. Chaput, Michel & Lederer (1961) have isolated another peptido-glycolipid, mycoside C_m, from a strain of *Mycobacterium marianum*. This compound contains only 6-deoxytalose and 3,4-di-*O*-methyl-L-rhamnose and differs further from mycoside C_1 in its peptide and lipid moieties. Nothing is yet known of the general composition of the glycolipids which contained the other sugars found in mycoside C, nor of that of the glycolipid from strain P-3 which contained xylose, glucose, rhamnose and a 6-deoxyhexose ether.

Mycoside C was originally recognized and defined as a species-specific substance on the basis of its characteristic infrared spectrum (Smith *et al.* 1957). Now that it appears that mycoside C is a mixture of glycolipids it must be considered possible that the similarity of the spectrum provided by preparations isolated from different strains does not indicate a constancy in the proportions of the glycolipids in these preparations but rather an insensitivity of the method of detection to variation in these proportions. On the one hand, preparation *A* contained only four of the eight sugars found in the other preparations and yet its infrared spectrum showed merely an intensification of those absorption bands common to these preparations (Fig. 1). Moreover, the mycoside C_m of *M. marianum* is reported to have an infrared spectrum practically identical with that of mycoside C (Chaput *et al.* 1961) and yet differs from mycoside C_1 in its lipid, peptide and carbohydrate moieties. On the other hand, a mycoside isolated from certain atypical mycobacteria (the J_{AT} of Smith *et al.* 1960*b*) showed distinct spectral differences from mycoside C although resembling mycoside C_m in possessing only two sugar components, very probably 6-deoxytalose and 3,4-di-*O*-methylrhamnose. It must be concluded that, although individual strains of *M. avium* appear to produce mixtures of glycolipids which possess the same monosaccharide units, it is possible that the glycolipids vary in their proportion from strain to strain, or even differ qualitatively with respect to their non-carbohydrate components.

Unlike the C-2 ethers of rhamnose and fucose that occur in mycosides A and B (MacLennan *et al.* 1961) the rhamnose and 6-deoxytalose ethers of mycoside C contain a C-3 methoxyl group. The L-enantiomorphs of 3-*O*-methylrhamnose (L-acofriose) and of 3-*O*-methyl-6-deoxytalose (L-acovenose) have been found previously in the cardiac glycosides (Tamm & Reichstein, 1951; Muhr, Hunger & Reichstein, 1954); but not in bacterial products. The optical configuration of the mycoside C sugars is unknown but mycoside C_m contains 3,4-di-*O*-methyl-L-rhamnose (Chaput *et al.* 1961).

Until recently (A. P. MacLennan, quoted by Smith *et al.* 1960*b*) 6-deoxytalose had not been found in bacteria although the L-enantiomorph has long been known as a cardiac glycoside component (Schmutz & Reichstein, 1951). It has now been isolated in crystalline form, but is of undetermined optical configuration, from mycoside C_m (Chaput *et al.* 1961) and as crystalline 6-deoxy-L-talose from the cell-wall carbohydrate of a strain of *Actinomyces bovis* (MacLennan, 1961). Its presence as a major component in this cell-wall carbohydrate and in the K antigen of *P. pseudomallei* raises the possibility that it may contribute to the serological

specificity of these substances. Among the sugars related to 6-deoxytalose that have been reported to occur in micro-organisms are D-talose, isolated from an antibiotic of *Streptomyces hygroscopicus* (Wiley & Sigal, 1958), and 6-deoxy-L-talosamine, isolated, together with its C-2 epimer, from the hydrolysis products of the capsular polysaccharide of Type V *Pneumococcus* (Barker, Brimacombe, How, Stacey & Williams, 1961).

SUMMARY

1. Eight sugars have been isolated from preparations of mycoside C, a mixture of glycolipids produced by strains of *Mycobacterium avium*.

2. The sugars of mycoside C were identified as glucose, arabinose, rhamnose, 3-*O*-methylrhamnose, 2,3- and 3,4-di-*O*-methylrhamnose, 6-deoxytalose and 3-*O*-methyl-6-deoxytalose.

3. One purified component of the glycolipid mixture contained only 6-deoxytalose, 3-*O*-methyl-6-deoxytalose, 3,4-di-*O*-methylrhamnose and a small amount of 3-*O*-methylrhamnose.

4. The 'K' lipopolysaccharide antigen of *Pseudomonas pseudomallei* also contains 6-deoxytalose.

The author wishes to thank Professor E. Lederer, Professor H. M. Randall and Dr D. W. Smith for the generous gifts of mycoside C which made this work possible. Thanks are also due to Professor H. M. Randall for recording infrared spectra, to Professor T. Reichstein for samples of sugars and to Dr A. M. Staub for a sample of 'K' antigen. The valuable technical assistance of Mr D. C. Hawkins and Miss V. Hart is gratefully acknowledged.

REFERENCES

Allen, S., Bonner, T. G., Bourne, E. J. & Saville, N. M. (1958). *Chem. & Ind.* p. 630.

Andrews, P., Hough, L. & Jones, J. K. N. (1955). *J. Amer. chem. Soc.* **77**, 125.

Barker, S. A., Brimacombe, J. S., How, M. J., Stacey, M. & Williams, J. M. (1961). *Nature, Lond.*, **189**, 303.

Bojalil, L. F. & Cerbon, J. (1960). *Amer. Rev. Tuberc.* **81**, 382.

Butler, K., Lloyd, P. F. & Stacey, M. (1955). *J. chem. Soc.* p. 1531.

Chambon, L., Bourdet, M. & Staub, A. M. (1957). *Ann. Inst. Pasteur*, **93**, 376.

Chaput, M., Michel, G. & Lederer, E. (1961). *Experientia*, **17**, 107.

Cifonelli, J. A. & Smith, F. (1954). *Analyt. Chem.* **26**, 1132.

Dische, Z. & Shettles, L. B. (1948). *J. biol. Chem.* **175**, 595.

Foster, A. B. (1952). *Chem. & Ind.* p. 1050.

Foster, A. B. (1953). *J. chem. Soc.* p. 982.

Gardiner, J. G. & Percival, E. (1958). *J. chem. Soc.* p. 1414.

Godin, P. (1954). *Nature, Lond.*, **174**, 134.

Hirst, E. L. & Jones, J. K. N. (1949). *Disc. Faraday Soc.* **7**, 268.

Hough, L., Jones, J. K. N. & Wadman, W. H. (1950). *J. chem. Soc.* p. 1702.

Jollès, P., Bigler, F., Gendre, T. & Lederer, E. (1961). *Bull. Soc. Chim. biol., Paris* (in the Press).

Krauss, M. T., Jäger, H., Schindler, O. & Reichstein, T. (1960). *J. Chromat.* **3**, 63.

MacLennan, A. P. (1961). *Biochim. biophys. Acta*, **48**, 600.

MacLennan, A. P., Randall, H. M. & Smith, D. W. (1959). *Analyt. Chem.* **31**, 2020.

MacLennan, A. P., Randall, H. M. & Smith, D. W. (1961). *Biochem. J.* **80**, 309.

MacLennan, A. P., Smith, D. W. & Randall, H. M. (1960). *Biochem. J.* **74**, 3P.

Muhr, H., Hunger, A. & Reichstein, T. (1954). *Helv. chim. acta*, **37**, 403.

Prissick, F. H. & Masson, A. M. (1957). *Canad. J. Microbiol.* **3**, 91.

Randall, H. M. & Smith, D. W. (1953). *J. opt. Soc. Amer.* **43**, 1086.

Runyon, E. H. (1955). *Amer. Rev. Tuberc.* **72**, 866.

Runyon, E. H. (1959). *Med. Clin. N. Amer.* **43**, 273.

Schmutz, J. & Reichstein, T. (1951). *Helv. chim. acta*, **34**, 1264.

Smith, D. W., Randall, H. M., Gastambide-Odier, M. M. & Koevoet, A. L. (1957). *Ann. N.Y. Acad. Sci.* **69**, 145.

Smith, D. W., Randall, H. M., MacLennan, A. P. & Lederer, E. (1960*a*). *Nature, Lond.*, **186**, 887.

Smith, D. W., Randall, H. M., MacLennan, A. P., Putney, R. K. & Rao, S. V. (1960*b*). *J. Bact.* **79**, 217.

Tamm, C. & Reichstein, T. (1951). *Helv. chim. acta*, **34**, 1224.

Trevelyan, W. E., Procter, D. P. & Harrison, J. S. (1950). *Nature, Lond.*, **166**, 444.

Wiley, P. F. & Sigal, M. V. (1958). *J. Amer. chem. Soc.* **80**, 1010.

Wong, S. & Weinzirl, J. (1936). *Amer. Rev. Tuberc.* **33**, 577.